

ANALYST:		VPDES NO	
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Parameter: *E. coli*
Method: Membrane Filter
02/06

METHOD OF ANALYSIS:

	HACH m-ColiBlue24® Broth (Standard Methods 18 th Edition is referenced by HACH)
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- 1) Is media stored in refrigerator at 2° to 8°C and protected from light? [7.1]
- 2) Is unopened media older than 1 year, discarded? [7.1]
- 3) Is labware, except when in metal containers, sterilized for at least 60 minutes in oven of sufficient size to prevent internal crowding and constructed to give uniform temperature of 170°C ± 10°C? [6.2]
- 4) If an autoclave is used, are items sterilized at 121°C for 15 minutes? [6.2]
- 5) Are pipettes in metal containers sterilized at 170°C for a minimum of 2 hours? [Permit]
- 6) Are all autoclaved materials identified with heat sensitive tape? [Permit]
- 7) Is sufficient room left in the sample container to facilitate mixing by shaking? [Permit]
- 8) Is glassware clean, free from cracks and chips, and properly stored? [Permit]
- 9) Are filtration units free from leaks and scratches? [Permit]
- 10) Are all procedures carried out in a manner that will neither inhibit desired bacterial growth nor cause contamination? [Permit]
- 11) Are all dilution bottles equipped with either glass stoppers or screw caps equipped with liners that do not produce toxic or bacteriostatic action upon sterilization? [SM 9030 B.13]
- 12) Is dilution water prepared with phosphate buffer and magnesium chloride solutions per Standard Methods? [SM 9050 C.1]
- 13) Is sample shaken vigorously, approximately 25 times prior to making each dilution and prior to filtration? [HACH instructions for Dilution Technique]
- 14) Are pipettes sized to deliver the required volume accurately and quickly? [Permit]
- 15) Are dilutions selected that will give 20 to 80 coliform colonies per plate? [12.2.3]
- 16) Are dilutions capable of showing permit excursions? Filtering of three different volumes – diluted or undiluted – is required. [9222.B.5.a]
- 17) If ampules are used, are they inverted several times to mix broth prior to pouring onto pad in petri dish? [HACH instruction -2]
- 18) Is sufficient media added to sterile absorbent pad (one ampule or 1.8 mL minimum) in a 50-mm MF petri dish for each test?? [11.1.1]
- 19) Is 0.45µm membrane filter placed in filtering apparatus grid side up? [HACH instructions #3]

Y	N

		Y	N
20)	Is small quantity of sterile buffered dilution water filtered to assure a good seating of filter prior to sample being filtered? [HACH Instructions # 5]		
21)	Is 100mL of sample or diluted sample poured into filter funnel? [HACH instructions #4]		
22)	If less than 20 mL of sample is used, is 10 mL of sterile, buffered dilution water added to funnel prior to applying vacuum? [HACH instructions]		
23)	Is vacuum applied after sample has been added to funnel? [HACH instructions #4]		
24)	Are three 20 to 30 mL portions of sterile, buffered dilution water used to rinse the funnel assembly following sample filtration? [11.1.2]		
25)	Is the filter placed on the media grid side up in a manner which prevents bubbles from being trapped under it? [HACH instructions & Permit]		
26)	Are forceps sterilized before each use by dipping in alcohol and flaming? [11.1.2]		
27)	Is filtration unit adequately rinsed between samples? [11.1.2]		
28)	Are filters incubated within 30 minutes following filtration? <i>DOCUMENTATION REQUIRED</i> [Permit]		
29)	Are petri dishes inverted and incubated in an air incubator for 24 hours at 35°C ± 0.5°C? <i>DOCUMENTATION IS REQUIRED</i> [6.1.1 & 11.1.2]		
30)	Is a sterile dilution water blank run after every ten samples? [9222 B.5.b]		
31)	Is a binocular microscope or a suitable optical device with 10 - 15X magnification used for colony counting? [6.1.4]		
32)	Are all colonies with any amount of blue color included in the plate count? (<i>E. coli</i> colonies may appear blue to purple.) [11.2.2]		
33)	Is <i>E. coli</i> density calculated correctly? [SM 9222 B.6.b]		
34)	Are plates with > 200 colonies (combined Total coliforms and <i>E. coli</i>) excluded from calculations? [HACH Instructions]		
35)	Is glassware tested annually for inhibitory residues or is the detergent certified by the manufacturer to be free of inhibitory effects? [SM 9020 B.3.a.2 and Permit]		
36)	Is each plate properly identified? [Permit]		
37)	Are plates sterilized prior to disposal? [Safety]		

PROBLEMS: